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A CONTRIBUTION TO THE SUBJECT OF THE FACTORS CONCERNED IN SOIL PRODUCTIVITY BY . . . . . *Conrad Hoffmann.*

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A Contribution to the Subject of the Factors Concerned in Soil Productivity.

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Plates XXIII to XXVII.

THE question as to the presence or absence in certain soils of toxic substances which exert a detrimental effect upon the growing crops is a matter of considerable importance. Ever since the time of de Candolle[1] there has existed a more or less extensive belief that crops excrete substances from their roots which are of a toxic nature. He it was who first attempted to explain certain phenomena associated with systems of crop rotation on the basis of plant-root excretions. He asserted that plants excrete from their roots substances that remain in the soil and injure other plants of the same species but do not necessarily affect plants of other species. In a crop rotation these toxic substances are supposedly destroyed during the period the other species of plants are grown, and so are removed before the original crop is again grown. In case, however, the same crop is continuously grown, these substances not only persist but accumulate, and sooner or later exert a detrimental effect with a resulting diminution in crop yields. In this way de Candolle thus early endeavored to ac-

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* Experimental work for this article was done in the laboratories of the Department of Agricultural Bacteriology of the College of Agriculture, University of Wisconsin, Madison, Wis.

1. De Candolle, Physiologie Vegetale, 1832, T. 111, p. 1474.

count for the better crop yields secured by rotation than by continuous one-cropping.

This explanation of de Candolle's has been largely retained up to the present time. As evidence of this one finds the following statement in a recent bulletin of the Bureau of Soils:[2]

"We must regard the excreta of growing roots as one of the main causes of the low crop yields obtained in improper crop rotation."

In other words, the toxicity which apparently exists in certain soils is thus attributed in large measure to the root excretions of plants. The work of Czapek and others, however, seems to indicate but little excretive activity on the part of plant roots. One is thus forced to look elsewhere for an explanation as to the source of these so-called toxins, if such exist in the soil.

A more frequent explanation of the diminishing crop yields is that the successive crops remove plant food, so that the subsequent crops have less available food and hence are obviously unable to produce such large yields. In connection with this latter explanation it is well to mention the investigations of Cameron, Whitney, Schreiner, Livingston[3] and others. Their work has been largely responsible for focusing research on the subject of soil toxicity as a cause of reduced soil productivity. According to them the reduced yields are not due to a lack of plant food, but rather to the presence of certain harmful substances in the soil solution. They believe that the concentration of the soil solution is practically the same in all soils.

Evidence both disproving and substantiating the statements of the above investigators relative to the presence of such soil toxins has been secured. It is an established fact that a deleterious effect upon crops is frequently exerted by an excess of certain mineral compounds. Of the latter, salts of magnesia, iron pyrites and sodium chloride are most frequently associated with such harmful effects exerted by soils on growing crops. The presence of excessive amounts of nitrates is likewise frequently responsible for such inhibitive action. This fact is well demonstrated by the work of Headden and Sackett.[4] They have shown that in certain localities of Colo-

2. Bulletin 40, Bureau of Soils, U. S. Dept. Agriculture, p. 40.

3. Bulletins 22, 23, 28, 30, 36, 40, 47, 80, Bureau of Soils, Dept. Agriculture.

4. Bulletins 155 and 160, Colorado Agricultural Experiment Station.

rado accumulations of nitrates have occurred which have resulted in a complete cessation of plant growth. The presence of various organic compounds has also been demonstrated to exert a harmful, if not a toxic action, at least when these substances are dissolved in the soil extracts. Schreiner[5] thus attributes to di-hydroxystearic acid a decided toxic property. He states that

"It would seem that di-hydroxystearic acid is either a direct or an indirect factor in the low productivity of soils."

Several other organic compounds have since been separated from the soil by him, which in liquid cultures exert a pronounced retarding influence upon wheat seedlings grown therein.

The source of these compounds has been variously attributed. Reference has already been made to the root excretions as the responsible agent for their origin. Beyond the evolution of CO_2 however, no evidence has been secured to indicate such excretions from plant roots. Czapek, for example, was unable even by the most sensitive and delicate methods, to demonstrate the presence of any organic acids in plant-root excretions. That these organic substances are formed as a result of the decomposition of the plant residues in the soil, due to the agency of soil bacteria, is an explanation of the problem that is relatively new but one which, because of its plausibility, is rapidly gaining support. Among the first to suggest this possibility was Dachnowski[6] in connection with his work on the growth of seedlings in peptone solutions inoculated with various soil organisms.

It is reasonable to assume that continuous cropping will deposit large quantities of plant tissue of a specific character in the soil. Further, that these tissues will undergo decomposition with the formation of by-products characteristic for the crop grown. Decomposition of wheat refuse undoubtedly will yield different by-products than clover or corn-stubble. Thus the continued one-cropping would tend to cause an accumulation of by-products which may prove toxic to the particular crop grown, whereas they may not affect other crops. By changing the rotation these would be destroyed so that when the original crop is again grown no toxicity would exist. An-

5. Schreiner, Bulletin 80, Bureau of Soils, U. S. Dept. Agriculture.

6. Dachnowski, Contributions 53, Botanical Laboratory, Ohio State University.

other possibility which presents itself is the fact that a specific type of by-products may tend to favor certain species of bacteria, which would cause a disturbance in the equilibrium of the soil flora. Brown[7] has very recently attempted to determine the influence of various crop rotation systems on the nature of the soil bacterial flora. He has shown that a two- or four-year rotation tends to maintain a higher ammonifying and nitrifying coefficient on the part of the soil than continuous one-cropping.

Another aspect to this problem of the reduction in crop yields due to continuous cropping is presented by the statements of Bolley,[8] in which he endeavors to explain the cause of a type of "soil sickness" prevalent in the north-central states of the Mississippi valley. Here repeated one-cropping has been largely practiced with wheat and flax respectively. The result has been a very marked decrease in the crop yields. This decrease seems hardly explicable on the basis of a rapid reduction in the supply of available plant food, although this is the explanation which has invariably been given for this phenomenon. Reference has already been made to de Candolle's explanation, namely that the reduction in crop yield is not due to a starvation, but rather to a poisoning occasioned by the accumulation of plant-root excretions. Bolley, however, claims that constant one-cropping permits the possibility of an accumulation, not of toxins, but rather of parasitic fungi and spores pathogenic to the crop constantly grown. On this basis, after repeated one-cropping, the soil becomes more or less extensively contaminated with such disease-producing fungi. Such a contamination with disease-producing factors would mean an increased percentage of sick and infected plants with a resulting diminution in crop yield. It is evident that Bolley does not attribute the reduced yield to a decrease in available plant food, and in this respect his explanation harmonizes with the view held by Cameron and Whitney,[9] who are responsible for the following statement:

"Soils are unproductive not because of the lack of the proper nutrients, but because they contain substances actually deleterious to plant growth."

7. Brown, Tech. Bulletin No. 6, pt. II, Iowa Agricultural Experiment Station.

8. Bolley, Science, Oct. 21, 1910.

9. Cameron and Whitney, Bulletins 22 and 40, Bureau of Soils.

Bolley as it were, explains the matter on the basis of an accumulation of disease organisms; Cameron and Whitney on the accumulation of deleterious waste products.

But to return to the work of the Bureau of Soils, more particularly the work of Schreiner. It is further claimed that the above deleterious substances are toxic and are the result of root excretions. Statements are thus made that "continuous cropping tends towards an accumulation of the waste products of plants, resulting in reduced crop yields"; that "these waste products are evidently of a soluble nature for when soil extracts are filtered through a Berkefeld filter, the toxic, retardative action of the soil persists in the filtrates"; evidence which would seem to indicate that the reduced yields are not directly due to the bacterial flora but rather to the chemical nature of the soil solution. The addition of lampblack and occasionally of $CaCO_3$ removes this toxic property, in which case seedlings grown in extracts so treated apparently make good growth. This evidence is indicative at least, that the retardative action is not due to a lack of plant food but rather to the presence of some chemical substance of a toxic nature which is removed from the sphere of action by the lampblack, and in some cases even by $CaCO_3$.

Considerable adverse criticism, notably that of King[10] has been directed against the methods employed by the Bureau of Soils for the attainment of these results. King claims that the quantities of the various toxic substances added to the soil filtrates in the Bureau's experiments are of such proportions that even the smallest amount used would be equivalent to 70 pounds per acre foot. If present in such quantities, he argues it should be an easy matter to isolate these substances from the soil, whereas, as a matter of fact, it is necessary to resort to the most elaborate methods to find even traces of these substances in the soils.

At this point reference should again be made to Bolley's hypothesis. If his explanation is correct, then filtration through a Berkefeld should remove the disease germs or their spores and thus render the filtrates innocuous so that they should support plant growth as efficiently as a sterilized filtrate from the same soil. As far as the author is aware this has not been tried out, but deserves further consideration before final acceptance of Bolley's hypothesis can be warranted.

10. King—Science, vol. 27, 1908, p. 694.

Greig-Smith[11] claims to have shown that volatile antiseptics are beneficial to such toxic soils. He claims that the former dissolve out and absorb the toxic ingredients and remove them from the sphere of activity, the so-called rhizoplane or root zone. He thus accounts for the beneficial action commonly secured when soil is treated with CS_2. Heating of soil as practiced in the partial sterilization of soil is likewise supposed to eliminate the toxic action or rather detrimental action manifested by such soils. Russell and Hutchinson[12] attribute the beneficial action of partial sterilization or antiseptification of soil to the destruction of certain forms of life which ordinarily prey upon the bacteria concerned in the conversion of unavailable plant food to available forms. They regard the protozoa, amœba and ciliates as competitive factors in the soil. If removed, the bacteria have full control and so can continue their multiplication with subsequent elaboration of plant food.

It is thus apparent from the above discussion that a multiplicity of hypotheses have been advanced to explain various phenomena manifested by soils which appear to give reduced crop yields. No doubt one can not attribute to any single factor the responsibility for the reduced productivity. Instead it appears far more probable that many factors contribute and cooperate to bring about aforesaid phenomena. In the light of the very complex nature of the soils, this seems the more probable and the only reasonable view to take relative to the problem in hand.

On the basis of the numerous explanations regarding this matter or closely related phenomena advanced by various investigators, it would appear that the soil bacterial flora must be regarded as a most important agent, responsible, at least in part, directly or indirectly, for the reduced crop yields on continuously cropped soils. Their action must undoubtedly center in their ability to decompose the various vegetable debris constantly being returned to the soil.

That the bacterial flora of the soil may be influenced by continuous one-cropping and that the bacteria in turn may influence subsequent crops, either the same or different, by the production of by-products harmful or beneficial to the plants, are matters which have as far as is known been largely ignored

11. Centbl. Bakt., 2 Abt., vol. 30, pp. 154, 156.

12. Russell and Hutchinson, Journal Agricultural Science, 1911, vol. 3, p. 111; 1912, vol. 5, pp. 27, 86; 1913, vol. 5, p. 152.

in considerations of soil toxicity problems. Very recently, however, Kellermann has called attention to the possible interrelationship existing between the soil bacterial flora and the growing crop.[13] It seems plausible to expect such an interreaction between the crops and the soil bacteria. We know how very sensitive bacteria are to slight variations in the composition of culture media. Whether in the cosmopolitan life of the soil the variations occurring in the composition of the soil due to the accumulations of crop residues may affect that life, has not been demonstrated, but is none the less most probable. Lyon and Bizzell[14] have suggested the influence of certain crops on nitrification which would thus influence subsequent crops.

Examination of soils in close proximity to the root systems of various plants reveals a marked difference in the quality as well as in the quantity of the bacteria in contrast to that found at some distance from the roots. (See Table I.) There is invariably a much higher germ content in the immediate vicinity of the roots. Thus of 32 determinations made on soils adjacent to and remote from the roots of various plants, all but five showed a much higher germ content near the roots than at some distance away. Totalling the 27 counts with an increase near the roots, one finds almost three times as many bacteria near the roots as at some distance therefrom. One must concede accordingly that the root system of plants exert a marked effect upon the bacterial flora of the soil in the immediate vicinity of the roots. Whether this influence is due to the respiratory products of the roots or to the products formed as a result of the decomposition of the root cells which are more or less constantly being sloughed off, the author is unable to say. Stoklasa has likewise shown that on the same soil under different crops the germ content shows marked variations. Thus:

Sugar beet soil	3-5 million.
Barley soil	1-2 million.
Clover soil	7-8 million.

The work herein reported endeavors to throw some light on this subject of reduced crop yields and soil toxicity. The author maintains that when a particular plant species is continually grown, the plant residues left in the soil occasion a change in the chemical composition of the soil, which in turn influences

13. Kellermann, Cir. 113, Bureau of Plant Industry, U. S. Dept. Agriculture.

14. Jour. Indust. and Engin. Chem., 5, 1913, No. 2, p. 136.

the bacterial flora. Certain plant species will favor certain types of bacteria and inhibit others and thus disturb the bacterial equilibrium of the soil. The new flora thus established then produces specific changes in the composition of the soil which affect subsequent plant growth, favoring some plant species and retarding others.

The problem as stated above is complex and presents many difficulties. With the three variable factors, crop, soil and bacteria, it was realized that little more than substantiation of the principles above set forth, in a few specific instances could possibly be hoped for. The establishment of the fact of such an interrelationship between soil crop and soil bacteria, was deemed worthy of an attempt.

On the basis of Schreiner's work, it was assumed that extracts made from sterilized soils in which different types of bacteria had been grown, would show differences when employed as nutrient solutions for the growth of various types of plant seedlings.

"The good or bad properties of a soil as shown by the growth of plants upon it, are largely transmitted to the aqueous extract of that soil."[15]

In the same manner a soil continuously cropped with one plant must show differences when employed in extract form for the growth of soil organisms.

"We must regard the excreta of the growing plant roots as one of the main causes of the low yields obtained in improper rotation of crops. By alternating the crops one invariably secures larger yields than where one crops continuously with a given crop."[16]

These two principles were employed as the basis for the work herein reported: First, to ascertain in individual cases what influence the growth of different pure cultures of soil organisms in sterile soil will have upon certain plant seedlings grown in the extracts of such soils; second, to ascertain what influence the growth of different plants in the same soil may have upon pure cultures of bacteria grown in the extracts of such cropped soils.

Considerable difficulty was experienced in the use of the ordinary perforated cork for the growth of the seedlings. After

15. Bureau of Soils, Bulletin 28, p. 21.

16. Bureau of Soils, Bulletin 40, p. 40.

some experimentation, the paraffin block method [17] was devised and subsequently used in all work herein reported.

Several preliminary experiments were performed in an endeavor to ascertain whether bacterial growth would modify the soluble chemical content of the soil solution as determined by the effect produced upon the growth of seedlings in the respective soil extracts. The general procedure employed was as follows:

For any given soil, 1000-gram portions of air-dry soil in large glass containers, were sterilized in the autoclave. In all cases one 1000-gram portion was left unsterilized to secure a control on the action of the normal soil flora. After cooling, the respective containers were inoculated with equal quantities of suspensions of the organisms employed, and, in addition, one with a suspension of the normal soil. The unsterilized soil sample received the same quantity of liquid used above, but in a sterile condition. Sufficient water was always added to give a moderately moist condition. The per cent employed was, of course, dependent upon the nature of the soil, relatively small quantities being employed for the sandy soils whereas the marsh and peat soils received a comparatively large per cent.

In addition to the above a sterile control for each soil was employed. This was made by adding to one container of sterilized soil, the same quantity of sterilized water which was employed in the preparation of the inoculating suspensions for the other containers.

The soil cultures thus prepared were incubated at room temperature for not less than seven days and usually for two to four weeks. To the cultures 1000 cc. of distilled water were then added. In the earlier work the mass was thoroughly and vigorously shaken and then filtered through a clean Berkefeld filter. In filtering it was found more expedient to add the soil-water mixture directly, rather than to attempt to siphon off the more or less clear supernatant liquid which collected on standing, and then filtering. Filtration was far more rapid. The soil added in the first method appeared to aid in the filtration and to retard the clogging-up of the Berkefeld. In the later work, however, it was found just as efficient to filter through ordinary filter paper, using Schleicher and Schüll's

17. C. Hoffmann, Botanical Gazette 55, No. 3, Mar. 1913. Centbl. Bact., 2 Abt., Bd. 34, 1912.

No. 588 folded filter for the purpose. The residue soils on the filter were washed with sufficient distilled water to make the filtrates up to 1000 cc. These were invariably clear, golden straw in color, and to all appearances would invariably be mistaken for ordinary bouillon. The extracts were then sterilized.

Five hundred cc. portions were then placed in pint Mason jars (a hydrometer cylinder being, however, a far more satisfactory container). In these were placed the paraffin blocks containing the plant seedlings. (Plate XXIII.) (The latter were secured in essentially the same manner as that employed by the Bureau of Soils.) The water cultures thus prepared were placed in the greenhouse to permit the growth of the plants. The water lost by evaporation and transpiration was restored in the form of sterile, distilled water.

All cultures were thus identical in every respect with the exception that different organisms had grown in the samples of soil from which the extracts had been made. Thus in the first series a field soil was employed. Of this 1000-gram portions were sterilized and then inoculated according to the following scheme:

1. Yellow ammonifier.
2. Ps. radicicola (garden pea).
3. Azotobacter.
4. B. prodigiosus.
5. B. liquefaciens fluorescens.
6. Cladothrix odorifera.
7. Aspergillus niger.
8. Sterilized soil.
9. Normal soil.
10. B. subtilis.

After 14 days' incubation, extracts of the respective soils were made according to method outlined above. These were then employed as nutrient solutions for the growth of wheat seedlings. The results are perhaps best apparent from Plate XXIV, which shows the condition of the seedlings after 18 days of growth. One observes a wide difference particularly in the root development of the seedlings in the various extracts. A most extensive root development occurred in the case of the extracts from the ammonifier, azotobacter and B. liquefaciens fluorescens. 1-3-5. (Plate XXIV.) In contrast to these, the

seedlings in the normal soil extract, and B. subtilis show a very sparse root development. It is suprising, however, that despite these pronounced differences in root development, there should be such slight differences in the developments of the tops.

In another test corn seedlings were employed with extracts from samples of black marsh soil in which the following organisms were grown:

1. Azotobacter.
2. B. denitrificans.
3. Proteus vulgaris.
4. Normal soil.
5. Cladothrix odorifera.
6. Yellow ammonifier.
7. Manure organism (B. mycoides?)
8. Sterile soil.

In this series Cladothrix odorifera and Proteus vulgaris occasioned a more luxuriant development in the case of corn and a marsh soil. In the case of the denitrifier the poorest development of the corn seedlings was secured. Here again the differential influence seemed to be confined more to the root than to the leaf system.

In this manner a large series of experiments (20) have been performed in which one or the other of the three factors involved has been modified. As typical of the results secured in these experiments, the data in tables II, III and IV are presented. One finds here marked variations both in the size of crop and in the dry weight of same as influenced by the various cultures. It is interesting to note that the manured soils (5 per cent finely ground dry manure) invariably show a larger gram yield than the unmanured soils; the two exceptions being in the case of corn and clover in the extracts made from the soil inoculated with Proteus vulgaris. If one measures the influence of the organisms by the weight of crop grown upon the extracts, one finds marked variations. In duplicate experiments where all three factors were identical, similar results are invariably secured, showing that whatever influence is exerted, the same is constant as long as the conditions remain the same. A change in any one of three factors occasioned a change in the influence exerted. Note the much larger yield of corn on the manured soil with B. prodigiosus in

contrast to that with Proteus vulgaris. The yield of clover with the yellow ammonifier on the manured soil is four times as great as that in the sterile manured soil. These are variations which are well beyond the factor of error. It is apparent that individual organisms when permitted to grow in pure culture in soils, influence the composition of the soil solution sufficiently to reveal an effect upon the crops grown in such solutions. On the supposition that continued one-cropping will modify the bacterial flora of the soil by favoring some forms and hindering others, it seems reasonable to expect that such a change of the flora will exert an influence upon subsequent crops.

THE EFFECT OF CROPS ON THE GROWTH OF ORGANISMS IN SOIL EXTRACTS.

Experiments were next performed in an endeavor to show that certain crops can so modify the soils' composition as to favor certain types of organisms and perhaps retard others. These were carried on in the following manner:

Three distinct types of soils, sand, loam and marsh, were employed. For each soil eight pots were filed with 3000 grams of soil. These were then planted to corn, oats and clover, using two pots to each soil for each crop; the fourth set being left unsown to serve as controls on the respective soils.

After luxuriant growth had occurred with penetration of the root systems throughout the soil mass (about six weeks' growth), the soil was shaken free from the roots, run through a ¼-inch sieve and then air-dried at 37° C. Of the dry soil so obtained, 1000-gram portions were vigorously shaken with 1000 cc. of distilled water and then immediately filtered through paper as previously described. In this manner between 600 and 800 cc. of clear extract were secured from each cropped soil. In the case of the marsh soil 1200 cc. of water were employed, owing to its large water-holding capacity.

The extracts thus secured were then measured with a burette into test-tubes in 10 cc. quantities. After plugging, these tubes were sterilized and subsequently used as culture media for the growth of pure cultures of soil bacteria. The rate of multiplication of the organisms in these extracts was used as an index of the influence, whether beneficial or detrimental, which the respective extracts exerted.

This was accomplished by making a suspension of a young culture of the specific organism under consideration in 100 cc. of sterile distilled water. After vigorous shaking, the faintly turbid liquid was filtered aseptically through glass wool, to insure a uniform and homogeneous suspension of the organisms. The 10 cc. quantities of the respective sterile soil extracts were then inoculated with these suspensions at the rate of 0.1 cc. per tube. For each extract six tubes were thus inoculated. By making germ content determinations on one tube a day, some idea as to the rate of multiplication of the organism in the respective soil extracts, could be secured. These determinations were made daily for a period of five days by which time most of the organisms employed had reached their maximum numbers. Plain gelatin was used as plating medium in some of the experiments, plain agar in others. The dilutions were so adjusted as to give plates upon which the colonies could be readily counted. In all cases an actual count of all the colonies on the plates was made, rather than estimating the total number by counting only a section of each plate.

In the tables which follow, Tables V to VII, the data secured in a few of these experiments are presented. These have been further arranged graphically in Plates XXV-XXVII which show more clearly the influence exerted by the various crops on the soil extracts. It will be noted that on the whole the results are consistent. This is very strikingly the case with the marsh soil (Plate XXV), where with all organisms employed the rate of multiplication was greatest in the corn-cropped soil, the other soils ranking as follows: clover-cropped, oat-cropped and control. This same order prevails with the three organisms here examined. Apparently the growing of the crops upon the marsh soil changed the composition of the soil solution in such a way as to enhance the growth of the bacteria in the extracts of the same.

With the loam soil, the growing of crops seems to have modified the soil solution so as to cause a decreased multiplication of the bacteria in the extracts in contrast to the control, which showed the most pronounced growth. With sand there is a very marked retardative influence evident, due to the cropping of the same with corn and oats. This applies to all three organisms. The normal in this case as with the loam, shows the most marked development with the exception of the ammonifier, where the clover-cropped soil exceeds the normal. In

all three cases, the normal and the clover-cropped soils show far more multiplication of the bacteria in their respective extracts than the corn or oat-cropped soils. On the basis of these data it appears as if the soil is the more important factor. The marsh soil when cropped enables a greater multiplicaton of the bacteria than the control or non-cropped soil. The reverse seems to be true with the loam and the sand soils here employed. The three organisms here experimented with are all similarly affected. Azotobacter, B. denitrificans, Sarcina lutea, B. liquefaciens fluorescens have also been used, but owing to contamination of the plates, the results have been ignored. It was possible, however, to note that on these plates the same relationship existed as above; invariably the corn-cropped marsh soil showed the most pronounced multiplication. In fact all the marsh soils which had been cropped showed in their respective extracts this stimulating effect on the growth of these organisms. In sand and loam, however, cropping appeared to exert a retarding influence on the growth of bacteria in the extracts from these soils.

In tables VIII to X further similar data are summarized which show the same general characteristics described above. They give added substantiation to the hypothesis previously stated, that growing crops modify the soil solution so that the latter exerts a more or less pronounced influence, stimulative or retardative, upon the bacterial species of the soil.

SUMMARY.

The data which have been secured in the experiments described above, enable one to make the following conclusions:

1. The growth of individual species of bacteria in a soil produces changes in the soil solution which manifest themselves by an increased or decreased development of plant seedlings when grown in extracts made from such soils.

Invariably this influence seems to affect the root development rather than the leaf development.

In repetitions of the same experiment consistent results are secured as long as all three factors, soil, bacterial species and crop, are the same. A change of any one factor modifies the results secured.

2. The growth of individual species of crops in a soil produces changes in the soil solution which manifest themselves by an increased or decreased bacterial multiplication in extracts made from such soils.

The extracts made from a marsh soil cropped by corn, oats or clover in all cases stimulated bacterial multiplication. This stimulation was consistently greatest in the case of the corn-cropped soil. In contrast to the marsh, the extracts from the cropped loam and sand soils invariably retarded the multiplication of the bacteria grown in the same.

3. There is a definite relation between the growth of crops in soil and the growth of bacteria therein. It is possible that one crop in a given soil may stimulate certain bacterial species which in turn will produce changes in the composition of the soil solutions. These changes may or may not influence beneficially subsequent crops.

Owing to the variability of the three factors involved, soil, crop and bacterial flora, it is impossible to establish any hard and fast laws for all cases.

It seems evident from the work that if these relationships could be established, one would have a definite basis on which to determine crop rotation systems. If corn favors the development of organisms whose growth stimulates oats, then oats can follow corn in a rotation; whereas, if corn retards the development of species favorable to oats or favors others detrimental to oats, then corn should not precede oats in a crop rotation.

Future work along the lines suggested by this paper should throw much light on this all-important subject.

TABLE I.—Showing influence of proximity to plant roots upon germ content of the soil.* Bacteria per gram.

Plant.	Adjacent to roots.	†Remote from roots one or more feet.
Currant	1,445,000	1,270,000 +
Asparagus	2,200,000	675,000 +
Grass	29,850,000	5,450,000 +
Iris	5,150,000	4,800,000 +
Raspberry	4,560,000	1,890,000 +
Rhubarb	3,600,000	970,000 +
Cabbage	8,085,000	740,000 +
Plum	1,335,000	595,000 +
Apple	3,200,000	1,345,000 +
Pea	1,770,000	1,520,000 +
Tomato	790,000	525,000 +
Rhubarb	1,035,000	465,000 +
Bean	63,500	59,500 +
Tomato	1,086,000	530,000 +
Sunflower	164,000	1,105,000 —
Potato	385,000	410,000 —
Potato	500,000	670,000 —
Cabbage	245,000	325,000 —
Cauliflower	213,000	504,000 —
Rhubarb	270,000	80,000 +
Tomato	565,000	80,000 +
Asparagus	84,000	66,500 +
Currant	275,000	177,500 +
Gooseberry	108,000	80,500 +
Hemp	6,500	500 +
Squash	930,000	114,000 +
Mangel	31,000	16,000 +
Tobacco	61,500	6,500 +
Strawberry	40,000	28,000 +
Cabbage	62,500	9,000 +
Grape	131,000	120,000 +
Cauliflower	72,000	40,500 +
Total	67,313,000	24,667,500

TABLE II.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of corn seedlings.

Organism.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.
Normal soil flora	22.5	25.0	47.5	1.665	17.5	25.0	42.5	1.652
Sterile soil	28.7	25.0	53.7	1.788	20.0	27.5	47.5	2.025
Cladothrix odorifera	32.5	27.2	54.7	1.356	12.5	25.0	37.5	1.610
Azotobacter	30.0	20.0	50.0	1.590	17.5	27.5	45.0	2.188
B. prodigiosus	20.0	20.0	40.0	1.560	23.7	25.0	48.7	2.237
B. proteus vulgaris	18.7	22.5	41.2	1.833	8.7	22.5	31.2	1.135
Ammonifier	20.0	21.7	41.7	1.304	17.5	25.0	42.5	1.946
Normal soil	20.0	20.0	40.0	1.264	15.0	30.0	45.0	2.153
B. denitrificans	32.5	21.2	53.7	1.311	20.0	27.5	47.5	2.105

* These results are compiled from data secured by Mr. B. Hammer and Mr. Eldredge.

† Greater number adjacent to roots indicated by +; less number by —.

TABLE III.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of wheat seedlings.

ORGANISM.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.
Normal soil flora	30.0	12.5	32.5	0.698	28.7	17.5	46.2	1.128
Sterile soil	22.5	13.7	36.2	0.750	27.5	17.5	45.0	1.060
Cladothrix odorifera	26.2	16.2	42.4	0.779	21.2	20.0	42.2	1.295
Azotobacter	27.5	15.5	43.0	0.700	23.7	17.5	41.2	1.151
B. prodigiosus	27.5	17.5	45.0	0.841	27.5	18.7	46.2	1.358
B. proteus vulgaris	25.0	15.0	40.0	1.038	27.5	18.7	46.2	1.171
Ammonifier	24.2	15.2	39.4	0.952	23.7	20.0	43.7	1.574
Normal soil	30.0	18.7	48.7	1.142	25.0	21.2	46.2	1.250
B. denitrificans	23.7	15.5	39.2	0.689	22.5	16.2	38.7	0.901

TABLE IV.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of clover seedlings.

ORGANISM.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.
Normal soil flora	10.0	1.2	11.2	0.109	13.7	4.3	18.0	0.190
Sterile soil	15.0	3.7	18.7	0.222	6.8	4.1	10.9	0.103
Cladothrix odorifera	16.2	3.1	19.3	0.320	12.5	6.2	18.7	0.446
Azotobacter	15.0	3.7	18.7	0.202	3.7	3.7	7.4	0.153
B. prodigiosus	16.2	4.3	20.5	0.188	8.7	3.7	12.4	0.340
B. proteus vulgaris	15.0	4.3	19.3	0.215	4.3	2.5	6.8	0.182
Ammonifier	15.0	4.3	19.3	0.110	12.5	6.8	17.3	0.543
Normal soil	7.5	1.2	8.7	0.194	12.5	6.8	19.3	0.434
B. denitrificans	12.5	4.3	16.8	0.179	8.7	5.6	14.3	0.441

TABLE V.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the ammonifying organisms. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh	Control	169,000	128,600	1,370,000	1,800,000	2,005,000
	Corn	167,500	455,000	4,000,000	66,400,000	25,000,000
	Oats	131,000	259,300	4,300,000	4,800,000	6,500,000
	Clover	127,500	2,060,000	7,000,000	16,100,000	11,400,000
Loam	Control	123,000	460,000	4,500,000	40,400,000	10,400,000
	Corn	139,000	2,175,000	5,700,000	11,900,000	11,900,000
	Oats	160,000	3,205,000	11,300,000	16,600,000	10,200,000
	Clover	137,500	810,000	4,400,000	6,200,000	5,300,000
Sand	Control	189,500	6,900,000	17,200,000	16,200,000	23,800,000
	Corn	118,500	112,000	570,000	325,000	5,600,000
	Oats	132,500	45,000	2,295,000	5,800,000	9,900,000
	Clover	175,000	7,300,000	31,400,000	31,400,000	21,200,000

TABLE VI.—Influence of the extracts of variously cropped soils upon the rate of multiplication of B. prodigiosus. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh	Control	238,500	59,000	234,000	420,000	4,900,000
	Corn	350,000	910,000	43,600,000	82,400,000	22,600,000
	Oats	286,500	57,000	387,000	2,210,000	11,500,000
	Clover	470,000	86,000	15,500,000	23,000,000	34,800,000
Loam	Control	231,500	900,000	21,700,000	19,700,000	17,400,000
	Corn	201,000	84,000	1,770,000	20,300,000	14,300,000
	Oats	302,000	80,300	1,380,000	8,700,000	13,300,000
	Clover	110,000	81,000	490,000	4,400,000	13,400,000
Sand	Control	287,000	5,200,000	45,300,000	38,200,000	16,000,000
	Corn	241,000	291,600	2,910,000	7,500,000	11,300,000
	Oats	510,000	22,000	268,000	6,700,000	8,600,000
	Clover	284,000	435,000	25,100,000	26,600,000	15,700,000

TABLE VII.—Influence of the extracts of variously cropped soils upon the rate of multiplication of B. proteus vulgaris. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh	Control	119,000	1,515,000	7,100,000	10,500,000	5,300,000
	Corn	107,500	9,600,000	13,900,000	43,200,000	43,300,000
	Oats	114,500	16,500,000	9,200,000	10,200,000	11,700,000
	Clover	109,000	17,500,000	24,700,000	26,900,000	18,800,000
Loam	Control	103,000	10,400,000	16,000,000	13,300,000	23,400,000
	Corn	118,000	9,000,000	15,600,000	14,200,000	12,700,000
	Oats	99,000	8,500,000	19,700,000	19,500,000	10,600,000
	Clover	108,000	1,250,000	10,100,000	8,800,000	8,100,000
Sand	Control	78,000	19,800,000	37,800,000	24,700,000	19,800,000
	Corn	80,000	7,500,000	7,100,000	10,000,000	8,800,000
	Oats	87,000	3,370,000	7,700,000	10,100,000	7,400,000
	Clover	95,000	13,200,000	14,700,000	16,400,000	11,300,000

TABLE VIII.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the ammonifying organisms. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh	Control	25,600	9,800,000	16,700,000	3,800,000	910,000	5,100,000
	Corn	81,000	9,000,000	97,000,000	137,000,000	14,400,000	65,000,000
	Oats	15,950	1,435,000	99,000,000	97,000,000	47,800,000	72,900,000
	Clover	40,900	930,000	61,000,000	115,000,000	106,000,000	57,000,000
Sand	Control	50,600	2,775,000	11,400,000	110,000,000	3,900,000	3,400,000
	Corn	124,500	3,340,000	14,400,000	.....	13,500,000	6,400,000
	Oats	95,000	2,170,000	7,800,000	4,040,000	150,000	260,000
	Clover	147,500	1,370,000	7,800,000	24,200,000	1,060,000	8,900,000
Loam	Control	100,000	3,560,000	22,500,000	6,300,000	8,000,000	21,300,000
	Corn	80,600	790,000	28,800,000	39,000,000	49,000,000	54,000,000
	Oats	26,400	1,315,000	20,600,000	16,400,000	8,400,000	25,700,000
	Clover	67,000	6,200,000	19,700,000	10,200,000	20,900,000	16,700,000

TABLE IX.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the denitrifying organisms. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh....	Control....	21,900	131,000	24,900,000	15,300,000	490,000	70,000,000
	Corn.....	18,300	53,000	50,400,000	101,600,000	41,200,000	70,000,000
	Oats.....	8,050	139,000	49,500,000	57,000,000	2,620,000	100,000,000
	Clover.....	11,550	226,000	32,000,000	61,700,000	24,100,000	70,000,000
Sand.....	Control....	13,200	197,000	44,800,000	36,900,000	90,000	35,500,000
	Corn.....	11,600	89,000	21,600,000	18,800,000	21,200,000	49,200,000
	Oats.....	16,700	.....	27,100,000	15,500,000	2,480,000	27,700,000
	Clover.....	17,700	101,000	39,900,000	29,600,000	23,600,000	27,200,000
Loam....	Control....	9,500	70,000	32,800,000	39,400,000	59,200,000	37,900,000
	Corn.....	9,100	29,000	49,000,000	49,800,000	55,800,000	38,900,000
	Oats.....	9,000	162,000	41,400,000	50,100,000	13,600,000	13,100,000
	Clover.....	9,800	1,500	28,800,000	23,700,000	30,300,000	15,200,000

TABLE X.—Influence of the extracts of variously cropped soils upon the rate of multiplication of Sarcina lutea. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh....	Control....	500	800	300	700	200	300
	Corn.....	300	300	10,600	42,000	90,000	148,000
	Oats.....	300	700	3,400	63,000	67,000	84,000
	Clover.....	400	600	1,300	24,900	68,000	93,000
Sand.....	Control....	500	200	7,100	16,700	500	28,100
	Corn.....	500	700	400	4,700	25,800	.....
	Oats.....	700	500	700	3,300	1,800	11,400
	Clover.....	500	300	700	7,200	11,700	20,300
Loam....	Control....	200	700	400	20,700	.....	32,400
	Corn.....	500	500	2,900	12,600	44,000	39,200
	Oats.....	400	400	300	16,100	15,600	35,300
	Clover.....	500	100	1,300	4,600	3,000	4,500